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Wildlife Toxicity Assessment for Hexachlorocyclohexane

APRIL 2009

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

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Acknowledgements

Key Technical Authors:	Christopher J. Salice, Ph.D.	US Army CHPPM Health Effects Research Program Aberdeen Proving Ground, MD 21010-5403
	George Holdsworth, Ph.D.	T N & Associates 124 S. Jefferson Circle Oak Ridge, TN 37830
	Christine A. Arenal, M.S Bradley E. Sample, Ph.D.	CH2M HILL 2485 Natomas Park Drive, Suite 600 Sacramento, CA 95833
Contributors:	William S. Eck, Ph.D. Mark S. Johnson, Ph.D.	US Army CHPPM Health Effects Research Program Aberdeen Proving Ground, MD 21010-5403
Outside Reviewers:	William W. Bowerman, Ph.D. Paul D. Jones, Ph.D. Phillip N. Smith, Ph.D.	Clemson University Michigan State University Texas Tech University

Point of Contact

For further information or assistance contact the following:

Michael J. Quinn, Jr., Ph.D.
U.S. Army Center for Health Promotion and Preventive Medicine
Toxicology Directorate: Health Effects Research Program
ATTN: MCHB-TS-THE, Bldg. E2100
Aberdeen Proving Ground, MD 21010-5403
(410) 436-1064/ DSN 584-1064
Michael.James.Quinn@us.army.mil

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Wildlife Toxicity Assessment for Hexachlorocyclohexane

CAS No. 608-73-1 **April 2009**

1. INTRODUCTION

Hexachlorocyclohexane (HCH) is a mixture of eight or more stereoisomers used as an insecticide to protect crops such as fruits and vegetables. Technical grade HCH (tech-HCH) is manufactured by the photochlorination of benzene, a process yielding a product of variable composition, typically containing 60-70% α-HCH, 5-12% β-HCH, 10-15% γ-HCH, 6-10% δ-HCH, plus some other minor components. Because of its synthetic relationship to benzene, tech-HCH has also been known historically as "benzene hexachloride," or BHC. Structurally, the stereoisomers differ only in the arrangement of the chlorine atoms on the six-carbon cyclohexane ring. α-HCH exists as a pair of enantiomers, while the other isomers are not optically active. Production of technical grade HCH in the U.S. was banned in 1976, but the substance may still be used in other countries, typically in small quantities (ATSDR 2005). Hexachlorocyclohexane and its component isomers are referred to by a number of scientific, trivial and proprietary names, many of which are listed in the Hazardous Substances Data Bank record for the compound (HSDB 2001). In this document, the acronym "HCH" will be employed to mean any formulation of hexachlorocyclohexane, without regard to the isomeric composition; where specific isomers are identified, the information pertains only to that isomer.

Lindane (CAS No. 58-89-9) is a purified preparation of γ-HCH, containing more than 99% of that specific isomer. The terms "lindane" and "technical HCH" should not be used interchangeably, as the isomers have different physical, chemical, and biological properties. Only γ-HCH (1α, 2α, 3β, 4α, 5α, 6β-hexachlorocyclohexane) has any appreciable insecticidal activity (IARC 1979). γ-HCH is used as a seed treatment for barley, corn, oats, rye, sorghum, and wheat. It is also used as a prescription medication for treatment of scabies and head lice in humans. In the past, γ-HCH was used in veterinary products to control mites, lice, and other pests, but no products are currently registered in the U.S. for this use. All the isomers are toxic to animals to varying degrees, and all have been shown to be persistent in the environment. (ATSDR 2005, Hegeman and Laane 2002).

Differential degradation and metabolism of HCH compounds results in non-uniform uptake by animal systems. α- and γ-HCH are found in highest concentrations in soil, air, and water. With respect to acute exposure for mammals, γ-HCH is the most toxic, followed by the α-, δ-, and β- isomers; for chronic exposure, β-HCH demonstrates the greatest toxicity, followed by α-, γ-, and δ-HCH (ATSDR 2005). Most research that has been done with the various HCH isomers has been focused on γ-HCH, however, because all isomers are found in the environment, it is appropriate to discuss each within this document.

This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of HCH on wildlife, identifying levels for the onset of toxicological effects, as described in reports of experimental studies of the compound. Evaluating the toxicity of HCH is aimed at establishing toxicity reference values (TRVs), which are values that can serve as protective exposure standards for wildlife ranging in the vicinity of contaminated sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Initial data search was conducted on June 6, 2001, as outlined in Appendix A. DIALOG was used to identify primary reports of studies and reviews on the toxicology of HCH. Separate searches were carried out linking the compound to laboratory mammals, birds, reptiles or amphibians (combined), or wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and "key words in context." All available abstracts of those articles selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For HCH, 175 articles were marked for retrieval from 1003 initial hits. Details of the search strategies and the results of each are documented in Appendix A. Secondary references and sources of information on HCH included the National Library of Medicine's Hazardous Substances Databank (HSDB 2001), an International Agency for Research on Cancer (IARC) monograph on HCH (IARC 1979), the U.S. EPA's Integrated Risk Information System (IRIS) (U.S. EPA 2001), and the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA 1997). An updated literature search was conducted on July 25-27, 2007, using a modified search strategy, also outlined in Appendix A.

2.2 Environmental Fate and Transport

All of the isomers of hexachlorocyclohexane have been detected in environmental media in the U.S. and throughout the world. While aerial dispersion of HCH has been prohibited, its widespread occurrence in airborne vapors and particulates has been demonstrated because the compound volatilizes

readily. Environmentally-collected samples of the HCH tend to not match the typical isomer distribution found in technical HCH (ATSDR 2005). Numerous studies (Nagata, et al. 2007; Phillips, et al., 2005; Padmakar, G.D., et al., 1994; Straub, G., 1991) have found that degradation of HCH isomers via microbial metabolism or catalytic activity of soils is possible. Microbial degradation of HCH isomers has been recently reviewed by Singh, et al. (2000).

The enantiomers of α-HCH are also selectively metabolized. Bidleman, et al. (2002) report that the (+)-enantiomer of α-HCH is preferentially metabolized in the Arctic Ocean, arctic lakes and watersheds, the North American Great Lakes, and the Baltic Sea. In contrast, in some marine regions (the Bering and Chukchi Seas, parts of the North Sea), the preference is reversed and (-)α-HCH is depleted. Apparent pseudo first-order rate constants in the eastern Arctic Ocean are 0.12 y^{-1} for (+)α-HCH and 0.030 y^{-1} for (-)α-HCH , and 0.037 y^{-1} for racemic α-HCH. These rate constants are 3-10 times greater than those for basic hydrolysis in seawater.

Levels of HCH isomers in air are thought to be dependent on temperature, with movements of α-HCH in cold Arctic air and γ-HCH in warm air from the lower United States having been observed. HCH concentrations in air of up to 50 ng/m^3 have been reported. Photodegradation makes a comparatively insignificant contribution to the removal of HCH from the air, the primary removal process being deposition to soil and surface water during rain-out (ATSDR 2005).

As might be expected for a pesticide intended primarily for the protection of crops, the soil serves as a primary repository for HCH, from which the compound does not readily migrate to other media. HCH is capable of leaching to groundwater, while low mobility and wind erosion can facilitate volatilization. Biodegradation is also a viable mechanism for HCH removal from the soil, because a number of bacteria, algae, and fungi have been shown to use HCH as a carbon source. Metabolic products of these processes include penta-, tetra- and tri-chlorinated cyclohexane and benzene compounds.

Biodegradation of HCH also can occur in surface water given a suitable microbial environment. By contrast, hydrolysis is a minor process for the compound in neutral conditions. Hexachlorocyclohexane and its component isomers have been detected in many surface water bodies in the U.S., with concentrations of up to 300 ng/L having been reported. Comparable concentrations have also been detected in groundwater samples in the U.S., although leaching from the soil is thought to be a comparatively slow process. Concentrations of up to 100 ng/L have been monitored in finished drinking water (ATSDR 2005).

The physical-chemical properties of the HCH isomers are summarized in the following table:

Table 1: Physical and Chemical Properties of HCH isomers.

Property	α-HCH	β-HCH	γ-HCH	δ-HCH
CAS No.	319-84-6	319-85-7	58-89-9	319-86-8
Molecular Weight (g/mol)	290.83			
Color	Brown to white	No data	White	No data
Physical State at 25°C	Crystalline solid	Crystalline solid	Crystalline solid; monoclinic prisms	Fine plates
Melting Point (°C)	159-160	314-315	112.5	141-142
Boiling Point (°C) at 760 mmHg	288	60 (at 0.5 mmHg)	323.4	60 (at 0.36 mmHg)
Odor	Phosgene-like	No data	Slightly musty	No data
Water solubility	69.5 mg/L; 10 ppm	5 ppm	17 ppm	10 ppm
Log K_{OW}	3.8	3.78	3.72	4.14
Log K_{OC}	3.57	3.57	3.0-3.57	3.8
Vapor pressure at 25°C	$4.5x10^{-5}$	$3.6x10^{-7}$ (20°C)	$4.2x10^{-5}$ (20°C)	$3.5x10^{-5}$
Henry's Law Constant at 25°C (atm-m^3/mol)	$6.86x10^{-6}$	$4.5x10^{-7}$	$3.5x10^{-6}$	$2.1x10^{-7}$
Conversion factors	1 ppm = 4.96 mg/m^3; 1 mg/m^3 = 0.2 ppm			

Source: All data from ATSDR (2005)

2.3 Summary of Mammalian Toxicology

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

A number of researchers have published oral median lethal dose (LD_{50}) values for isomers of HCH or for one or more technical grade formulations. For example, Gaines (1969) surveyed the acute lethality of 98 pesticides and 2 metabolites of DDT in Sherman rats, deriving values for γ-HCH (lindane) of 88 and 91 mg/kg in males and females, respectively. Boyd and Chen (1968) reported an LD_{50} of 184 mg γ-HCH/kg in male Wistar rats fed a normal protein diet and 157 mg/kg in others fed standard laboratory chow. These values contrasted with an LD_{50} of 95 mg/kg in rats receiving a protein-deficient diet. Truhaut et al. (1974) calculated an oral LD_{50} value of 100 mg/kg for Wistar rats (sex unstated) receiving a

single oral dose of γ-HCH in olive oil. Golden hamsters (with an LD_{50} of 360 mg/kg) appeared to be less sensitive to the acute toxic effects of the compound. In a range-finding experiment prior to studying the immunomodulatory effects of γ-HCH in Swiss and Balb/C mice, Meera et al. (1992) reported an acute oral LD_{50} of 120 mg/kg for γ-HCH, while Lahiri and Sircar (1991) obtained an LD_{50} for the γ-isomer of 86 mg/kg in female Swiss mice. Lewis (1992) documents some of the above values and provides other oral LD_{50} values for γ-HCH as follows: 76 mg/kg in rats, 44 mg/kg in mice, 40 mg/kg in dogs, 60 mg/kg in rabbits, and 127 mg/kg in guinea pigs.

Although the above data show a reasonable consensus on the range of LD_{50}s for γ-HCH in experimental animals, other researchers have studied the acute oral lethality of technical grade HCH with strikingly different results. For example, Dikshith et al. (1989) gavaged Swiss mice, Wistar rats, and white rabbits (strain unstated) with technical grade HCH in peanut oil and obtained LD_{50} values of 1,435, 1,752.9, and 1,362.4 mg/kg, respectively. However, the content of the γ-isomer in their technical grade formulation was only 10.6%, with relative proportions of the α- and β-isomers of 72.4% and 11.9%, respectively. Krishnakumari et al. (1982) reported an LD_{50} of 4,174 mg/kg in male and 5,673 mg/kg in female CFT-Wistar rats for "technical X-factor", a mixture derived from technical HCH by removing most of the β- isomer. Taken together, these data suggest either enhanced toxicity of γ-HCH compared to that of the other isomers, or antagonistic effects of other isomers when combined.

There are a number of studies in which laboratory animals have been exposed to HCH acutely while investigating endpoints other than lethality. For example, Grabarczyk et al. (1990) gavaged male rabbits (strain unstated) with multiple doses of 7 mg/kg ($1/10^{th}$ of the LD_{50}) γ-HCH for either (1) a total of five doses during a period of a week, (2) as (1) but with a subsequent 1-week recovery period, or (3) a total of 20 doses over a 4-week time period. Blood samples were taken at weekly intervals to measure hematological parameters, and histopathological evaluations of liver and kidney were made of all animals at term. Although no changes were observed in either the hemoglobin level or hematocrit in any group of tested rabbits, a comparative increase in reticulocyte count was observed in those rabbits receiving γ-HCH for a 4-week duration. This group also displayed a statistically significant decrease in leucocyte count and an increase in the number of lymphocytes bearing micronuclei. Some histopathological effects were also evident in this group of rabbits, including hepatocellular damage and degeneration of some glomeruli and tubular cells of the renal cortex.

In a study of mechanistic significance to the renal toxicity of γ-HCH, Dietrich and Swenberg (1991) gavaged between five and seven F344 rats/sex/group and male NBR rats/group with 10 mg/kg-day γ-HCH in corn oil for 4 days. The researchers studied the histopathology of the kidney and attempted to localize any $\alpha_{2\mu}$-globulin formation using immunohistochemical techniques. All male F344 rats receiving

γ-HCH displayed renal hyaline droplet formation and the presence of $\alpha_{2\mu}$-globulin, whereas neither of these effects was observed in female F344 or male NBR rats. Furthermore, gas chromatographic separation and analysis of kidney extracts from these animal models suggests that γ-HCH can bind to low molecular weight components extracted from male F344 rats, while those from female F344 or male NBR rats showed no trace of such substances. The authors concluded that hyaline droplet-related renal disease, induced by the action of γ-HCH as well as a number of other environmental toxicants, is associated with $\alpha_{2\mu}$-globulin formation in those animal models that have the capacity to synthesize this unique protein (such as male F344 rats). More recently, Hong and Boorman (1993) evaluated the hematopoietic effect of γ-HCH in mice using a bone marrow progenitor cell assay. Male $B6C3F_1$ mice were gavaged with 20 or 40 mg γ-HCH/kg in corn oil for 3 days or 0, 10, or 20 mg/kg for 10 days. Apart from a decrease in weight of the thymus, there was no difference in comparison to controls in body or organ weights after γ-HCH for 3 days. The thymus also showed some signs of atrophy on histopathological examination. Mice receiving 10 or 20 mg γ-HCH for 10 days showed a statistically significant decrease in spleen and thymus weights, but an overall increase in the weight of the brain. When colony-forming units in granulocyte-macrophage progenitor cells were assayed 2 days after the final 3-day treatment at 20 or 40 mg/kg γ-HCH, their numbers were significantly less than those from untreated mice, although colony formation returned to normal values after a 14-day recovery period. *In vivo* γ-HCH activity also resulted in a reduction of colony-forming units in the spleen. This reduction coupled with other experimental findings suggested to the authors that myelotoxicity may be a sensitive endpoint in γ-HCH-treated mice.

2.3.1.2 Mammalian Oral Toxicity – Subacute

A number of studies on the subacute toxicity of HCH to laboratory animals have been conducted. For example, Srinivasan et al. (1984) exposed male Wistar rats to 800 ppm dietary β- or γ-HCH for 15 days and observed metabolic and histopathological changes in the kidney of treated animals. In this study, kidney dysfunction was marked by the appearance of excess urinary glucose and creatinine concentrations (both isomers), and by increased urinary urea concentration (γ-HCH only). By contrast, urinary protein levels were lower than controls in those rats exposed to γ-HCH. Histopathological evaluation of the kidneys from HCH-treated rats revealed major changes in the architecture of renal tubule cells, their severity being greater in the kidneys of those animals receiving the β-isomer. This caused some degenerative changes in renal tubular epithelium. The dietary level associated with this effect approximates a dose of 40 mg/kg-day, assuming a default food consumption rate for rats of 0.05 kg food/kg body weight (BW)-day. γ-HCH was also etiologically linked to histopathological lesions of the liver and kidney when administered by gavage at 20 mg/kg-day to male Wistar rats for 15 days (Tiwari et al. 1982). The researchers also measured the activities of a number of serum enzymes and monitored

fluctuations of liver lipids in response to insecticide alone or to insecticide plus supplemental L-ascorbic acid (200 mg/kg-day). However, there is uncertainty surrounding the toxicological significance of the biochemical and physiological changes described in these experiments.

Cornacoff et al. (1988) evaluated the immunotoxicity of β-HCH by exposing female $B6C3F_1$ mice to the compound for 30 days at dietary concentrations of 0, 100, or 300 ppm. Animals were monitored for body weight changes; ovarian, uterine, and splenic histopathology; antigen-specific IgM and IgG plaque formation; and proliferative responses of splenocytes to the mitogens phytohemagglutinin (PHA), concanavalin A (con-A), and B-cell mitogen lipopolysaccharide (LPS), among other end points. The only compound-related changes noted were the reduced proliferation of splenocytes in high-dose mice in response to LPS, PHA, and con-A, and the number of peripheral blood leucocytes. Therefore, the 100 ppm dose level, which is equivalent to 15 mg/kg-day (assuming food consumption of 0.15 kg food/kg body weight) represents a possible no-observed-adverse-effect level (NOAEL); the lowest-observed-adverse-effect level (LOAEL) would be 45 mg/kg-day.

Ravinder et al. (1990) exposed male Swiss mice to dietary supplements of 400 or 800 ppm technical HCH or 200 or 400 ppm γ-HCH for 2 weeks and assessed the resulting fluctuations in blood and organ lipid content. The striking dose-dependent increases observed in relative liver and kidney weights were accompanied by dramatic increases in serum lipids, especially triglycerides. Moreover, the overall extent of these changes was greater in those mice receiving technical HCH than in those receiving γ-HCH. Histopathological examination of pieces of excised liver revealed instances of hepatomegaly in those animals exposed to either dietary level of technical HCH. Taking the changes in relative organ weights as the most sensitive toxicological parameter under investigation in this study, LOAELs of 60 mg/kg-day technical HCH and 30 mg/kg-day γ-HCH are suggested from the data in the study, if a default food consumption rate of 0.15 kg food/kg BW-day is assumed for the mice employed in the study.

Barros et al. (1991) looked for changes in hepatic lipid peroxidation-related parameters after administering α- or γ-HCH-supplemented diets to male Wistar rats for 15 or 30 days. Liver supernatants were assayed for thiobarbituric acid reactants (TBARs), while liver microsomes were measured for cytochrome P-450, NADPH-cytochrome P450 reductase activity, and the production of superoxide radicals. The activities of glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase also were measured in liver supernatants. Although no histopathological changes to the liver were observed as a result of the treatments, some statistically significant changes in the biochemical balance of the liver were observed, such as increases in the level of cytochrome P450 after either treatment or duration and increases in catalase activity in rats exposed to the α-isomer. These changes allowed the suggestion that a toxicological NOAEL for α- and γ-HCH would be lower than the dietary concentration of 20 ppm (approximating a dose of 1 mg/kg-day assuming a food

consumption rate of 0.05 kg food/kg BW-day) if the biochemical changes are precursors of subsequent toxicological effects.

Lahiri and Sircar (1991) evaluated changes in the structure and function of the adrenal gland in female Swiss mice gavaged with γ-HCH in olive oil at various concentrations and different durations up to 3.07 mg/kg-day for 6 weeks or 2.86 mg/kg-day for 8 weeks. They measured the levels of glucocorticoid in the plasma and adrenal at term and monitored the adrenal gland for compound-related changes in organ weight, histopathology, and altered concentrations of cholesterol, ascorbic acid, and glucocorticoid. The authors interpreted the biochemical fluctuations as either compound-induced depression of corticosteroid activity or a suppression of the enzymes that catalyze the peripheral transformation of steroids. Histopathological changes in the adrenal gland were characterized by marked atrophy of the cortical region and progressive disorganization of the cellular cords. However, it is difficult to identify a NOAEL and LOAEL from this study because histopathological examinations do not appear to have been done on members of all experimental groups.

Behavioral and possibly compound-related neurochemical changes were monitored in male Wistar rats after 0 or 10 mg/kg γ-HCH in olive oil was administered via gavage in 25 daily doses over a 29-day period (Llorens et al. 1992). Animals were assessed for behavioral signs, maze behavior, active avoidance, and, on necropsy, changes in regional concentrations of biogenic amines and metabolites in the brain. Although rats treated with γ-HCH showed an increase in spontaneous motor activity, no additional behavioral or neurochemical perturbations were found.

2.3.1.3 Mammalian Oral Toxicity - Subchronic

A substantial number of studies on the toxicology of HCH have focused on histopathological lesions in the liver and their potential for subsequent progression to form tumors. For example, Ito et al. (1975) reported hepatic tumors in male Wistar rats exposed to diets containing up to 1,500 ppm of various HCH isomers for periods of time up to 72 weeks. When animals were necropsied, the livers were weighed, examined macroscopically, then processed for histopathological examination. Most of the toxicological consequences of treatment were confined to the rats receiving α-HCH, with histopathological lesions in the liver observed at all exposure levels and durations. Moreover, rats exposed to 1,000 ppm α-HCH or greater for 48 weeks and beyond showed an increasing incidence of "nodular hyperplasia" and hepatocellular carcinoma. In a subsequent study, Nagasaki et al. (1975) attempted to assess the universality of their α-HCH/liver tumor effect by exposing other strains of mice and other species of experimental animals to dietary supplements of 500 ppm α-HCH. The compound induced nodular hyperplasia and hepatocellular carcinomas in the livers of mice but not in those of male rats (Wistar) or Syrian golden hamsters. Among various strains of mice tested, DDY mice showed the highest incidence

of liver nodule formation, the lowest being in C57BL/6 mice. A similar conclusion was reached by Tryphonas and Iverson (1983) who exposed randomly bred black male (HPBC57BL) mice to diets supplemented with 500 ppm α-HCH for various periods of time up to 50 weeks. Animals were necropsied and examined for gross lesions in the liver and lungs. The authors gave a very detailed description of histopathological lesions of the liver that appeared from 3 weeks onward. Adenomatous nodules became apparent after 31 weeks, but no carcinomas were evident at any of the time intervals employed in the study. The authors concluded that HPBC57BL mice were much less susceptible to hepatocellular carcinoma formation than DD mice.

The extent to which the HCH-induced liver lesions are unique to mice was explored by Barros and Saliba (1978) who exposed 10 Wistar rats/group (sex unstated) to 0, 0.9, or 900 ppm technical HCH (58.3% α- and 7.57% γ-HCH) in the diet for 90 days. Blood samples were taken at termination to measure glucose, and excised livers were weighed, measured for glycogen and glucose-6-phosphatase activity, and examined histopathologically along with samples of brain, kidney, and spleen. A number of histopathological lesions of the liver and kidney were observed including degeneration and the appearance of a vacuolated cytoplasm in the liver, and enlarged "nephrocytes" with the appearance of vacuoles and cytoplasmic granules in the kidney. Assuming a default food consumption rate of 0.05 kg food/kg BW-day in this strain of rat, the dietary levels would equate to a LOAEL of 45 mg/kg-day, with a NOAEL of 0.045 mg/kg-day.

The γ-isomer of HCH has been the subject of intense toxicological scrutiny. For example, Dikshith et al. (1978) gavaged 16 male rats/group (strain unstated) with 0 or 17.6 mg/kg-day in peanut oil for 90 days. At sacrifice, liver, kidney, testis, epididymis and brain were excised for biochemical, morphological, and histopathological evaluations. The toxicological effects of γ-HCH were marked by the onset of necrotic changes to the liver and the formation of testicular lesions, implicating 17.6 mg/kg-day as an unbounded LOAEL for the observed effects in this study.

Shivanandappa and Krishnakumari (1981) mixed technical grade HCH (72% α-, 5% β-, 13.6% γ-, and 8% δ-HCH) with laboratory chow to give concentrations of 0, 100, 250, 750, 1,500, and 3,000 ppm (equivalent to 0, 5, 12.5, 37.5, 75, and 150 mg/kg-day). These preparations were made available to male CFT Wistar rats for 90 days, at which point the liver, kidneys, heart, lungs, spleen, brain, adrenals, and testis were excised for weighing and histopathological processing. While all the members of the high-dose group died, survivors in the lower groups displayed a number of histopathological lesions, including hypertrophy of the liver and adrenals at 750 ppm and above, and cellular changes to the liver at a threshold concentration of 250 ppm. Perhaps the most sensitive indices of HCH toxicity were the increases in relative liver, heart, and kidney weights that showed statistically significant differences at 100 ppm, which was the lowest dietary concentration of HCH employed in the study compared to controls.

This suggests LOAELs for these effects of 5 mg/kg-day, the dose equivalent to the dietary concentration of 100 ppm.

The IRIS record for γ-HCH describes an experimental study by the Zeocon Corporation (1983) that is not generally in the public domain (U.S. EPA 2001). However, as described by the IRIS compilers, incremental dietary additions of 0, 0.2, 0.8, 4.0, 20 or 100 ppm γ-HCH to 20 Wistar KFM-Han (outbred) SPF rats/sex/group for 12 weeks resulted in liver hypertrophy, as well as a range of kidney effects including tubular degeneration, interstitial nephritis, and hyaline droplets formation. Dietary concentrations of 4.0 ppm γ-HCH induced few if any of these responses, thereby justifying this dietary level as equivalent to a NOAEL. According to the IRIS record, food intake data in the original report allow a dose of 0.29 mg/kg-day to be calculated for the males and 0.33 mg/kg-day for the females (U.S. EPA 2001). Assuming the food intake to be constant throughout the treated groups, this would point to respective LOAELs of 1.45 and 1.65 mg/kg-day (averaged to 1.55 mg/kg-d), equivalent to the dietary supplementation of 20 ppm.

In an effort to link the toxicological and biochemical effects induced by HCH, Oesch et al. (1982) fed both sexes of susceptible mice (CF1) and non-susceptible rats and mice (Osborne-Mendel and B6C3F1) with γ-HCH-spiked feed for either 3 days or 3 months. Feed concentrations in the 3-day studies were 0, 51, 124, and 266 ppm γ-HCH, and those in the 3-month studies were 0, 56, 111 (170 in B6C3F1 mice), and 360 (270 in B6C3F1 mice) ppm. The researchers monitored relative changes in the weights of major organs such as the liver, and measured the hepatic activities of hepatic 7-ethoxycoumarin-O-dealkylase, epoxide hydrolase, glutathione-S-transferase, and UDP-glucuronosyltransferase for correlation to the morphological changes. As described by the authors, γ-HCH feeding for 3 months was associated with an increase in the relative liver weights of both sexes of CF1 mice and of female Osborne-Mendel rats at the highest insecticide dose. No equivalent effects were seen in B6C3F1 mice, none of which survived at the highest dose level. A number of liver microsomal and supernatant enzymes showed dose-dependent alterations in activity due to γ-HCH, but there was no clear demarcation of effects between those susceptible and non-susceptible strains as defined by their toxicological responses to γ-HCH. Given standard default food consumption rates for rats and mice of 0.05 and 0.15 kg food/kg BW-day, respectively, the relative liver weight data equate to LOAELs of 18 mg/kg-day in Osborne-Mendel rats and 54 mg/kg-day in CF1 mice. Corresponding NOAELs would be 5.6 mg/kg-day and 16.65 mg/kg-day, respectively.

As mentioned above, the "technical X-factor" is a β-HCH depleted preparation that also has been tested for toxicological activity in experimental animals. For example, Muralidhara and Krishnakumari (1983) fed male CFT-Wistar rats for 90 days with dietary concentrations of 0, 10, 50, 250, 750, 1,500, or

3,000 ppm, approximately equivalent to doses of 0, 0.4, 2, 11, 32, 64, and 135 mg/kg-day. The content of technical X-factor in these experiments was 16% α-, <1% β-, 16% γ-, 50% δ-HCH, and 16% other components such as ortho- and trichlorobenzenes. The authors monitored body weight changes, food consumption, clinical signs, organ weights at necropsy, the activity of serum enzymes, and histopathological changes. Increases in the relative weights of liver, kidney, and testes in animals receiving 750 ppm and above were observed. Histopathological changes in these organs were also evident at the same concentration, for example, hepatocellular hypertrophy and hyperplasia. Taken together, these data point to a LOAEL of 31.8 mg/kg-day and a NOAEL of 10.8 mg/kg-day for the hepatic and renal effects of technical X-factor. Conversely, Van Velsen et al. (1986) administered 0, 2, 10, 50, or 250 ppm β-HCH in feed to 10 Wistar rats/sex/group for 13 weeks and observed a similar range of toxicological parameters to those described by Muralidhara and Krishnakumari (1983) for technical X-factor (depleted in β-HCH). For example, 5/10 high-dose males and 6/10 high-dose females died as a result of treatment, while those animals receiving lower doses exhibited a number of sub-lethal responses to β-HCH including increases in relative kidney and liver weight; changes in hematological parameters; histopathological effects on the liver, kidney, and spleen; and toxicological impacts to thymus, adrenal cortex, testes, ovaries, and endometrium. The most sensitive toxicological responses appeared to be the increases in relative kidney and liver weights, allowing the designation of a LOAEL for the former response of 0.1 mg/kg-day, and 0.5 mg/kg-day for the latter. This would justify the designation of 0.1 mg/kg-day as a NOAEL for the alterations in relative liver weight due to β-HCH.

Changes in calcium metabolism and toxicological impacts to the kidney were the primary focus of a study by Andrews and Gray (1990), who gavaged male Long-Evans rats with 0, 10 or 20 mg γ-HCH/kg-day for 10 weeks. They monitored serum chemistry parameters; the appearance of metabolites in urine; various parameters of bone morphometry such as femur volume, density, strength, flexibility, etc.; and the appearance of histopathological lesions in kidney. None of the indicators of bone morphometry changed as a result of exposure to HCH, the primary toxicological effects were alterations to kidney structure and function. Along with an increase in the relative organ weight, there was an increase in lactate dehydrogenase activity in the urine, and histopathological lesions were evident in the stained sections of the kidney, including droplet formation. Since the latter responses were observed in animals receiving either dose level, 10 mg/kg-day may be considered an unbounded LOAEL for the kidney effects observed in this experiment.

2.3.1.4 Mammalian Oral Toxicity - Chronic

One of the earliest definitive reports of the toxicological effects of HCH in experimental animals was that of Fitzhugh et al. (1950) who exposed 10 Wistar rats/sex/group to diets supplemented with 10, 100 or

800 ppm α-, β-, and γ-HCH. Other groups received 50 ppm α-HCH, 5, 50, 400, or 1,600 ppm γ-HCH and 10, 50, 100, or 800 ppm technical HCH. Exposures of 0, 5, 10, 50, 100, 400, 800, or 1,600 ppm correspond to daily doses of 0, 0.25, 0.50, 2.5, 5.0, 20, 40, and 80 mg/kg-day assuming a default food consumption rate of 0.05 kg food/kg BW-day. The technical HCH employed in the experiment consisted of 64% α-, 10% β-, 13% γ-, 9% δ-, and 1.3% , γ-HCH. Body weights and food consumption were monitored weekly, and most animals were allowed to live out their natural life span. Although no differences in food consumption rates were observed, body weight gain (or growth) was significantly reduced in the 80 mg γ-HCH /kg-day (1,600 ppm) and 5 mg β-HCH/kg-day (100 ppm) dose groups during the first 6 months of the study (from weaning). These dose groups may be considered LOAELs for this effect. Corresponding NOAELs are 40 mg/kg-day (800 ppm) for γ-HCH and 2.5 mg/kg-day (50 ppm) for β-HCH. Absolute and relative liver weights were monitored at sacrifice or termination, the threshold exposure rates showing good agreement when putative NOAELs and LOAELs for changes in relative liver weight were compared to those associated with the onset of histopathological lesions. For example, the most severe hepatic effects were obtained in rats receiving β-HCH at 10 ppm, suggesting a LOAEL of 0.5 mg/kg-day, assuming a default food consumption rate of 0.05 kg food/kg BW-day. Equivalent NOAELs and LOAELs for the hepatic effects of the other isomers and technical HCH would be, for the NOAELs, 0.5 mg/kg-day for α- and technical HCH, and 2.5 mg/kg-day for γ-HCH. Candidate LOAELs would be 2.5 mg/kg-day for α- and technical HCH, and 5 mg/kg-day for γ-HCH. The histopathological effects of HCH were far less profound in the kidney than in the liver, and higher doses of HCH were required to induce testicular atrophy among male Wistar rats. For the latter effect, the NOAELs were 0.5 mg/kg-day for β-HCH and 5 mg/kg-day for α- and technical HCH, with corresponding LOAELs of 5 mg/kg-day for β-HCH and 40 mg/kg-day for α- and technical HCH.

The histopathological effects of HCH described by Fitzhugh et al. (1950) did not explicitly address the compound's potential to induce tumors in the main bodily organs of their chosen animal model, Wistar rats. However, many of the subsequent long-term toxicological studies of HCH have focused primarily on this toxicological consequence, which although is not appropriate for TRV derivation when taken alone, can be very helpful as part of a weight-of-evidence when combined with other endpoints more likely to affect fitness and cause population level effects. NCI (1977) carried out a dietary supplementation study of γ-HCH in 50 Osborne-Mendel and B6C3F1 mice/sex/group, with exposure for 80 weeks followed by a further observation period of 29-30 weeks in rats and of 10-11 weeks in mice. Time-weighted average (TWA) concentrations of γ-HCH in feed were 0, 236, and 472 ppm in male rats; 0, 135, and 270 ppm in female rats, and 0, 80, and 160 ppm in both sexes of mice. In comparison to 10 concurrent controls/sex/group, treated rats displayed no changes in body weight gain, few if any clinical

signs, no obviously treatment related non-neoplastic histopathological lesions, and no incipient tumor formation. By contrast, the authors described an increased incidence of hepatocellular carcinomas in male mice that was statistically significant at the low dietary concentration (80 ppm). However, the tumor incidence in mice receiving the higher dose of HCH was not significantly different from controls, thereby calling into question the extent to which the tumors in low-dose mice were related to treatment.

Another of the older long-term exposure studies of γ-HCH in experimental animals involved the administration of the compound in the diet to four beagle dogs/sex/group at concentrations of 0, 25, 50, or 100 ppm for 104 weeks (Rivett et al. 1978). The researchers monitored a full suite of toxicological responses, including daily clinical signs, food intake twice weekly, and body weights weekly. Blood samples were obtained prior to exposure and after 4, 13, 26, 52, and 102 weeks to measure hematology and clinical chemistry parameters. Animals were also subjected to periodic urinalysis, ophthalmoscopy, electroencephalogram readings, and a complete necropsy and comprehensive histopathological examination of the major organs. A supplemental group of animals (4/sex) were exposed to 200 ppm γ-HCH for 32 weeks. As calculated by the authors, the doses equivalent to the dietary concentrations of γ-HCH were 0, 0.83, 1.6, and 2.92 mg/kg-day, at which levels no compound-related effects on body weight gain, food consumption, ophthalmology, hematology, urinalysis or histopathology were observed. However, the authors noted that animals exposed to 100 ppm γ-HCH (2.92 mg/kg-day) showed a slightly darkened liver and a statistically significant increase in serum alkaline phosphatase activity after 52 and 102 weeks (and after 32 weeks in those exposed to 200 ppm γ-HCH). These results suggest a LOAEL for the enzymological and hepatic effects of γ-HCH may be 2.92 mg/kg-day, with a NOAEL of 1.6 mg/kg-day. However, this assumes that the change in serum enzyme activity has toxicological significance.

Technical HCH has also been the subject of long-term toxicological investigations, such as Kashyap et al. (1979) who exposed 30 Swiss mice/sex/group to 100 ppm technical HCH (68.7% α-, 6.5% β-, and 13.5% γ-HCH) in the diet for 80 weeks. In other phases of the experiment, the researchers gavaged other mice at a dose of 10 mg/kg-day for 80 weeks, and carried out a skin painting study in which 0.25 mg technical-grade HCH was applied to the dorsal skin of mice in 0.1 ml olive oil twice a week for the same duration. Perhaps the most striking effect of these treatments was the development of tumors in the liver in significantly greater numbers than in control mice. The tumors were marked morphologically by the development of a rough surface and the appearance of yellow nodules. Histopathologically, a sequence of lesions was evident, starting with the onset of oval cells, proliferation of the bile duct, hypertrophy of the parenchymal cells, development of hepatoma, and then the formation of hepatocellular carcinomas. In a similar experimental protocol, Munir et al. (1983) exposed both sexes of Swiss and Balb/c mice, Wistar rats, and Syrian golden hamsters to 500 ppm technical HCH (proportions of isomers not stated) in the diet, with additional dietary concentrations of 125 and 250 ppm technical HCH in male Swiss mice.

The duration of exposure was for up to 22 months. Progressive tumor formation was evident in the livers of both strains of mice, with 76% of male Swiss mice and 19% of females developing adenomas after 8–11 months of exposure. However, there was a 100% incidence of hepatocarcinomas after 12–14 months of exposure. The authors found that no tumors developed if treatment was discontinued after 2–3 months, but that hepatic tumor incidence reached 92% if initial exposure was for 3 months or more before discontinuing. Similar results were obtained in Balb/c mice, but not in the rats and hamsters used in the experiment.

Dikshith et al. (1991) evaluated the dietary toxicology of technical HCH in 20 male "albino" rats/group in which the expressed aim was to define a chronic NOAEL. The isomeric composition of the technical grade employed in the studies was 72.43% α-, 11.95% β-, 10.62% γ-HCH, and 2.5% other substances. Animals received dietary supplementation of 0, 0.5, 5, 25, 250, or 500 ppm for a year, equivalent to respective doses of 0, 0.04, 0.4, 2.0, 20, and 40 mg/kg-day, as calculated by the authors. There were no clinical signs or mortality in animals exposed at the lowest dose, but increases in relative liver weight were noted in those exposed to 25 ppm (2.0 mg/kg-day) and above. Histopathological changes to the liver, kidney, and testis were apparent in those rats exposed at 20 mg/kg-day or more. Therefore, 2 and 20 mg/kg-d are considered the respective NOAEL and LOAEL for histopathological measures. Based on the data in their study, the authors suggested that a technical HCH concentration of 0.5 ppm in feed would be a NOAEL for technical HCH, an equivalent dose being 0.04 mg/kg-day. However, it is unclear which toxic effect they are assuming to be the driver for such a designation. If the increase in relative liver weight is considered to be the most sensitive toxicological effect of HCH in this animal model, the next highest dose of 0.4 mg/kg-day would be a candidate NOAEL, with a dose of 2 mg/kg-day representing the LOAEL for the onset of this toxicological response.

In a more recent study, Shouche and Rathore (1998) fed two groups of Swiss mice either 500 ppm technical HCH (containing 6.5% γ-HCH) for 100 days or 10 ppm for 400 days. Although clinical chemistry fluctuations and some histopathological changes in the liver were apparent, the primary focus of the study was on the compound-induced toxicological effects on the heart, which become enlarged during treatment and was marked histopathologically by hypertrophic degeneration of the aorta and myocardium.

2.3.1.5 Mammalian Oral Toxicity - Other

A substantial number of toxicological studies of HCH have examined the compound's capacity to disrupt the reproductive and developmental characteristics in experimental animals or to induce teratogenic effects. Although exposure durations of these studies are generally short, they were conducted during a sensitive life stage (i.e., gestation) with related endpoints (e.g., reproductive and

developmental parameters); therefore, data collected from these studies are as valuable as those from chronic studies for TRV value derivation (USACHPPM 2000). Among those studies in which HCH was administered to pregnant animals during gestation, gavage doses of γ-HCH of 5, 10, or 20 mg/kg-day to New Zealand white rabbits on gestation days (GDs) 6–18 and to CFY rats on GDs 6–15 had no effect on any reproductive, developmental, or teratogenic parameter investigated (Palmer et al. 1978). Likewise, gavage doses of 0, 6.25, 12.5, or 25 mg/kg-day of "Benesan," a formulation of HCH isomers consisting of approximately 50% γ-HCH, had no obvious reproductive, developmental, or teratological effects when administered to pregnant female Wistar rats on GDs 6–15 (Khera et al. 1979). Negative results for these effects were also obtained in pregnant ITRC rats receiving gavage doses of 20 mg/kg-day γ-HCH on GDs 6–14 (Saxena et al. 1986). However, when this regimen was coupled with a concurrent administration of normally benign 100 ppm cadmium acetate in drinking water, a range of deleterious reproductive, developmental and teratogenic consequences was obtained, including a reduction in the number of pups, reduced ossification of the skull, and an increased incidence of fused sternebrae and wavy ribs.

Beard and Rawlings (1998) administered lindane (γ-HCH) to three generations of mink at a rate of 1 mg/kg-d in their feed. While no overt signs of toxicity were noted and the pesticide did not reduce the number of mink mated, lindane treatment reduced both the proportion of mink that were subsequently born ($P < 0.1$) and the litter size of mink that were born ($P < 0.05$). Testis size was reduced in the lindane-treated third generation males ($P < 0.05$). Exposure to lindane from conception resulted in a decrease in reproductive efficiency when mink were subsequently mated, leading to a 40% reduction in the mean number of kits born. Data from what appears to be the same study (Beard et al. 1997) also found a decrease in the percentage of females accepting a second mating, yet mean litter size was not affected.

Reproductive and developmental toxicological studies were also carried out on Wistar rats by Srinivasan et al. (1991), who, in a complex protocol, exposed pregnant females to dietary β-, or γ-HCH at concentrations of 0, 50, 200, 400, and 800 ppm (0, 2.5, 10, 20, and 40 mg/kg-day assuming a default food consumption rate of 0.05 kg food/kg BW-day) during either gestation alone, gestation followed by lactation, or lactation alone. In monitoring pup survival for up to 28 days after birth, the researchers observed considerable maternal toxicity (i.e., all animals died) in those rats exposed to 800 ppm β-HCH, while those receiving 400 ppm produced a near normal number of litters. However, all the pups died after 5 days, an effect that was partially evident (48% survival) in the pups of dams receiving 200 ppm β-HCH. Additionally, some sporadic toxicological consequences were seen in weanlings whose dams were exposed to HCH during lactation. Based on the observed pup toxicity, a dose level of 20 mg β-HCH /kg-day could be considered the LOAEL, with a corresponding NOAEL of 10 mg β-HCH /kg-day for this effect. For γ-HCH, no effects on female fertility or pup survival were observed at levels up to 400 ppm.

Thus, 20 mg γ-HCH/kg-day would be the NOAEL and 40 mg γ-HCH/kg-day (the dose level at which reduced fertility and increased pup mortality occurred) would be the LOAEL.

Matsuura, et al. (2005) reported a study using male and female Crj:CD(SD)IGS rats (SPF) receiving 0, 10, 60, or 300 ppm 99.5% pure γ-HCH (corresponding to 0, 0.5, 3.0, and 15 mg/kg-d, assuming a default food consumption rate of 0.05 kg food/kg body weight-day) for two generations of animals. Reduced body weight gain and food consumption were observed in both F0 and F1 animals, and females died at the 300 ppm level. At 10 and 60 ppm, pathological examination revealed increased liver weights and centrilobular hepatocellular hypertrophy in both sexes. Increased kidney weights and increased hyaline droplets in the proximal tube epithelium were observed in males receiving 10 ppm of greater γ-HCH, without any noticeable histological changes. Neonatal toxicity was observed in both sexes and generations, including suppressed body weight gain at 60 and 300 ppm. The postnatal survival rate in F2 offspring was decreased due to lack of maternal behavior in dams at 300 ppm.

As with the previously described endpoints, there is evidence that mice are more susceptible than rats or rabbits to the reproductive, developmental, and teratogenic effects of HCH and its isomers. For example, Sircar and Lahiri (1989) gavaged pregnant female Swiss mice with γ-HCH in either early (GDs 1–4), mid- (GDs 6–12), or late (GDs 14–19) pregnancy, and monitored reproductive and developmental deficits in formed fetuses (the first two groups) or pups (the last group). γ-HCH administered in early pregnancy appeared to have the ability to prevent implantation, an effect that was reversible by administering estrogen. The compound administered in mid-pregnancy was associated with 100% resorption, despite the appearance of a normal number of implantation sites. Pups born to dams exposed to γ-HCH in late pregnancy died within 12 hours (high dose group) or 5 days (low dose group) of parturition. Employed concentrations were 0.09, 0.15, 0.17, or 0.26 mg/animal, depending on treatment group. For the early pregnancy group, the dose was 0.26 mg/animal-day, or 10.7 mg/kg-day over four days. The mid-pregnancy dose was 0.15 mg/animal-day, or 6.14 mg/kg-day, over seven days. The doses that were administered over 6 days in late pregnancy were 0.09 mg/animal-day and 0.17 mg/animal-day (3.58 mg/kg-day and 7.16 mg/kg-day). Because all the tested concentrations elicited reproductive/ developmental deficits, only an unbounded LOAEL may be derived. The lowest dose associated with observed effects was 3.58 mg/kg-day.

No reproductive or developmental/teratogenic effects were observed when pregnant Swiss mice received a single gavage dose of 0, 5, 25, 50, 100, or 200 mg technical HCH/kg in peanut oil on GD 9 (Dikshith et al. 1990). However, the same authors reported in an abstract that mice (strain unstated) exposed to the same doses during GDs 6-12 displayed maternal toxicity at the two highest doses and reproductive deficits at the lower doses (Srivastava et al. 1991). Evidence of fetal resorption and a reduction in the number of fetuses/litter suggested a LOAEL of 50 mg/kg-day and a NOAEL of 25

mg/kg-day for these toxicological consequences. These findings were amplified in a subsequent full-scale reproductive/developmental study in which pregnant female Swiss mice were gavaged with either 0, 5, 25, or 50 mg/kg-day technical HCH (72.43% α-, 11.95% β-, and 10.62% γ-HCH and 2.5% other compounds) in peanut oil during either GDs 2–6 or 6–12 (Srivastava and Raizada 1993). All fetuses examined were free from teratogenic effects, although some appeared to have subcutaneous hemorrhages. One sign of toxicological significance was a reduction in the number of fetuses/dam in those high-dose mice exposed during the post-implantation phase (GDs 6–12). These data lend support to the threshold dosimetry emerging from the author's earlier study. Additionally, maternal weight gain during gestation was significantly reduced in the two highest dose levels, suggesting a LOAEL of 25 mg/kg-day and a corresponding NOAEL of 5 mg/kg-day for this effect.

Hassoun and Stohs (1996) carried out a reproductive/developmental toxicity study in which pregnant female C57BL/6 and DBA/2J mice were exposed to a single gavage dose of γ-HCH of either 30 or 45 mg/kg on GD 12. The doses chosen caused 14 and 25 percent maternal deaths and an increase in the number of dead and resorbed fetuses in high-dose C57BL/6J mice, but not in DBA/2J mice. However, they did not observe developmental abnormalities such as those observed in parallel exposures to 2,3,7,8-tetrachloro-p-dibenzodioxin (2,3,7,8-TCDD). The latter effects, cleft palate and hydronephrosis, are thought to be mediated through the aryl hydrocarbon receptor (AhR), allowing the suggestion, by default, that such a mechanism is inoperative in the toxicological effects of HCH. Given the maternal and fetal toxicity observed at 30 mg/kg-day, this dose level may be considered an unbounded LOAEL.

A report by Lakkad et al. (1982) is one of several in which male experimental animals were exposed to HCH and impaired reproductive performance was observed. In this case, male Swiss mice were exposed to 500 ppm technical HCH in the diet for 4, 6, or 8 months, then mated with untreated females. The latter were necropsied in mid-pregnancy to monitor the number of implants, dead embryos, percent fertility, and other reproduction parameters. Among the key results were significant decreases in the number of live embryos/female compared to those mated with untreated males. This finding was manifested for all exposure durations. In another approach, Dalsenter et al. (1996) exposed 10 male Wistar rats/group via gavage to either 6 mg γ-HCH/kg in peanut oil for 5 days (equivalent to 1.2 mg/kg-day) or a single dose of 30 mg γ-HCH/kg. Observed effects included reduced numbers of spermatids in the latter group 2 weeks after treatment, with some histopathological changes. The same research group reported relative reductions in testicular weight, serum testosterone, and sperm and spermatid counts in the male offspring of female Bor: spf, TNO rats exposed to 1 or 6 mg γ-HCH/kg while lactating [postnatal days (PND) 9-14] (Dalsenter et al. 1997a). These represent dose levels of 0.17 and 1 mg/kg-day. The researchers reported some alterations of the structural architecture of the testes of the male offspring and some subsequent changes in reproductive performance and sexual behavior. Dalsenter et al. (1995, 1997b) also examined

the reproductive performance and mating behavior of male Wistar rats exposed to HCH through their mothers who received a single oral dose of 30 mg γ-HCH/kg on GD 15. A key observation was the apparent reduction in sexual performance/activity in treated offspring, a finding apparently correlated with a decline in serum testosterone concentration. However, when mating did occur, their reproductive performance was the same as in controls (male progeny of unexposed dams).

Two-generation study investigating the oral toxicity of lindane, as well as other selected substances, was carried out in Sprague-Dawley rats (Yamasaki et al. 2005). Rats were exposed to either 0, 10, 60, or 300 ppm lindane in feed at five weeks of age (for F0 parents and 3 weeks of age for the F1 parents) and for 10 weeks prior to and during the mating period, through gestation, delivery days, and during lactation (0, 0.5, 3, 15 mg/kg-d, respectively). Observation included clinical signs (e.g. body mass, feed consumption) and other specific parameters (e.g. sperm characteristics, hormone levels, estrous cycles, vaginal opening and prenuptial separation of the penis). No obvious effects were found in any of the parameters in either the F0, F1, or F2 offspring. Few other details were reported.

The impact of HCH on testicular structure was the focus of a study in male Swiss mice that were exposed to a 500 ppm dietary supplement of technical HCH for 30 to 40 weeks (Nigam et al. 1979). Six animals/group were terminated at monthly intervals to follow the progression of any macroscopic or histopathological lesions that developed. The authors reported an increase in the relative weight of the testis after 3 months of exposure when compared to the zero time controls, and described a concurrent suite of histopathological changes marked by degeneration of the seminiferous epithelium and damage to spermatogonic cells. Given a default food consumption rate of 0.15 kg food/kg BW-day for this animal model, the 500 ppm dietary exposure would equate to a dose of 75 mg/kg-day, and represent an unbounded LOAEL for the testicular effects observed.

Gautam et al. (1989) and Chowdhury and Gautam (1990) also presented evidence of technical HCH's ability to induce changes in the architecture of the male reproductive system by daily gavage exposure of 12 male Charles Foster rats to 0, 3, or 6 mg/kg in gum acacia for 180 days. Animals receiving either dose of compound displayed a reduction in body weight gain compared to controls and histopathological degeneration of both the muscular layer of the vas deferens and the seminiferous tubules. This result indicates a dose of 3 mg/kg-day as a LOAEL for the induction of changes to the male reproductive system of Charles Forest rats by technical HCH.

There are two studies that examined the reproductive/developmental toxicology of HCH after dosing males and females for an extended period prior to mating. These include (1) a limited three-generation reproductive study of dietary technical HCH (doses of 125 and 250 ppm or 6.25 and 12.5 mg/kg-day) in Druckrey rats (Srivastava and Raizada 2000), and (2) a 90-day study in which both sexes of rat (strain unstated) were exposed to γ-HCH by gavage at 0, 10 or 20 mg/kg-day for 80 to 90 days prior to mating

(Gray et al. 1988). In the first experiment, there were no effects of HCH on such reproductive/ developmental parameters as pregnancy rate, gestation duration, pup weights, or malformations and variations, although elevated levels of HCH were detected in important target organs such as adipose tissue. This suggests that a dose of 12.5 mg/kg-day (equivalent to the highest employed dietary concentration of 250 ppm) may be a suitable NOAEL for the compound's reproductive/developmental effects. In the second experiment there were few, if any, affected reproductive parameters in treated females, but all the high-dose and many of the low-dose progeny died shortly after birth. This led the authors to speculate that the deaths were due to a maternal factor, related to either lactation or a lack of maternal care (Gray et al. 1988).

Seiler et al. (1994) examined the reproductive/developmental toxicity of γ-HCH on New Zealand white rabbits in which females were first exposed to 0.8 mg, 3 times/week for 12–15 weeks prior to artificial insemination, then throughout gestation. Uteri were examined on GDs 1, 6, and 11 with a range of reproductive indices being monitored, although there were no obvious differences in any parameter when treated and control animals were compared.

An experiment by Wolfe and Esher (1980) was particularly relevant to wildlife because the design included an 8 month dietary exposure to 200 ppm γ-HCH in trapped and/or bred old-field mice (*Peromyscus polionotus*) or cotton mice (*Peromyscus gossypinus*) maintained as breeding pairs. No differences were observed between treated and control groups in the number of litters, their size, or the number of surviving offspring. Consequently, a LOAEL could not be identified from the results of this study. As no effects were observed at the 200 ppm γ-HCH level (39.1 mg/kg-day for Old-Field mice and 24.4 mg/kg-day for Cotton mice), this dose was considered to be an unbounded NOAEL.

Three additional toxicity studies evaluating the effects of HCH or its isomers on mammals were located. In the first, the cytogenetic properties of γ-HCH were examined using an in vivo assay for chromosomal abnormalities in the bone marrow cells of Swiss mice exposed by gavage for 7 days to 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, or 2.4 mg/kg-day (Kumar et al. 1995). The authors considered 1.6 mg/kg-day to be a no-effect-limit-dose (NELD), but do not provide the rationale for this selection. Based on the onset of the statistically significant effect compared to controls (as presented in the paper), a dose of 1.0 mg/kg-day appears more defensible. The corresponding LOAEL would be 1.2 mg/kg-day.

The biochemical and physiological changes associated with, and possibly etiologically linked to, the toxicological effects of HCH have not been worked out with any certainty. However, in one novel experimental approach, Wolff and co-workers (Wolff et al. 1987, Chadwick et al. 1987) monitored hepatic tumor formation in genetically linked hybrids, specifically obese mottled yellow (Avy/a), lean pseudoagouti (Avy/a), and lean black (a/a) mice. Yellow mice developed far more combined hepatic

adenomas and carcinomas than did black mice, with pseudoagouti mice being intermediate. The authors interpreted their data as implicating a role for both the Avy gene and obesity in the development of hepatic tumors induced by γ-HCH.

Studies were conducted to further investigate the mechanism whereby lindane is suspected to cause embryonic lethality in vivo. Scascitelli and Pacchierotti (2003) exposed female mice to three daily oral doses of either 15 or 25 mg/kg-d either before or immediately following mating to understand whether embryotoxic effects were occurring pre or post implantation. Although changes were found in the number of two-cell degenerating embryos, # blastomers/morula, and 40% reduction in mitotic index, no statistically significant effect was observed in number of litters/female.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Similar to other organochlorine pesticides, the liver appears to be a primary target organ of HCH. This organ responds to the chemical insult with biochemical and physiological changes that appear as (1) clinical chemistry fluctuations, (2) morphological changes indicative of a toxic response, and (3) the formation of a sequence of non-neoplastic and neoplastic histopathological lesions in some animal models. Considerable variability exists as to which species and strains of experimental animals are susceptible to hepatic tumor formation induced by HCH. Many of the compound's tumorgenic effects have been described in Swiss mice, although DDY and Balb/c mice also appear to be susceptible. By contrast, C57BL/6 mice may be comparatively resistant to the tumorgenic effects of HCH. In addition to the liver, adverse effects were observed in the kidneys, male reproductive organs, heart, as well as the endocrine and reproductive systems.

The results related to tumorgenic effects of HCH and the candidate threshold doses for the onset of the compound's non-neoplastic toxicological effects indicate that there is some variability in an animal's response to the different isomers of HCH. For example, the early experiments of Nagasaki and co-workers suggest that the α- form of the compound is the most toxic of the HCH isomers. Although these studies may be useful in examining the relative toxicity of the HCH isomers or formulations, tumor formation is an endpoint of unknown ecological significance and one not generally used to derive a TRV. Therefore, studies that only evaluate tumor formation were not considered sufficient for TRV derivation. As shown in Figure 1, the LD_{50}s associated with γ-HCH are more than an order of magnitude lower than those reported for the isomeric mixture, technical HCH, supporting the trend observed in the tumorgenic data. However, ranking the LOAELs for the hepatic, renal, and reproductive toxicity of technical HCH and its isomers does not reveal a consistent pattern of differential toxicity among the HCH preparations.

A key feature of the compound's renal toxicology is the appearance of $\alpha_{2\mu}$-globulin-related nephropathy in male rats exposed to the compound. However, this response is not universal, even among male rats, since male NBR rats neither produce the required protein nor exhibit the consequent nephropathy (Dietrich and Swedberg 1991). Nonetheless, $\alpha_{2\mu}$-globulin-related nephropathy may be a sentinel toxicological effect for wildlife species that are capable of synthesizing this protein.

A number of lines of evidence implicate HCH as a toxicant to the male reproductive system, with histopathological changes to the testis and vas deferens resulting from exposure of mice to HCH via gavage (Gautam et al. 1989, Chowdhury and Gautam, 1990). This response is also a feature of the toxicological effects of other organochlorine pesticides such as chlordane, with both compounds inducing changes that correlated with consequent reproductive deficits (Lakkad et al. 1982). Effects on female reproduction (e.g., prevention of implantation [Sircar and Lahiri 1989], fetal resorptions [Srivastava et al. 1991 and Srivastava and Raizada 1993], or reduced fetuses/dam [Srivastava and Raizada 1993]) and on pup survival (Sircar and Lahiri 1989) have also been observed. However, concentrations at which male and female reproductive effects become apparent are greater than those associated with systemic toxicity (e.g., morphological changes to the liver).

Table 2 and Figure 1 provide tabular and graphical displays of the range of available NOAELs and LOAELs for the observed effects. The HCH data represent three Orders and three Families of *Mammalia* including Rodentia: Muridae, Carnivora: Canidae and Mustelidae, and Lagomorpha: Leporidae. Test animals include multiple strains of laboratory mice and rats, rabbits, dogs, and two wild species, the old-field mouse and cotton mouse. Generally, the studies are of moderate to high quality with several NOAELs and/or LOAELs for various endpoints of effect and various organ systems identified. Therefore, the studies presented below are considered suitable for the derivation of wildlife TRVs.

2.3.2 Mammalian Inhalation Toxicity

No data are available on the toxicology of HCH via inhalation.

2.3.3 Mammalian Dermal Toxicity

The report by Dikshith et al. (1989) examined the single dose median lethality (LD_{50}) for technical HCH when applied dermally and obtained values of >8,000 mg/kg in Wistar rats and 1,786.3 mg/kg in white rabbits. There appeared to be consistent though relatively small differences in lethality according to whether the solvent vehicle was acetone or dimethyl sulfoxide (DMSO).

A long-term skin painting study of technical HCH in Swiss mice did not detect a statistically significant increase in tumor incidence compared to solvent-treated controls (Kashyap et al. 1979).

2.4 Summary of Avian Toxicology

2.4.1 Avian Oral Toxicity

2.4.1.1 Avian Oral Toxicity – Acute

Krishnakumari and Muraldhara (1981) investigated the acute oral toxicity of technical HCH (71% α-, 6.5% β-, 13% γ- 7.0% δ-, and 1.5% ε-HCH) and technical X-factor in the domestic hen, through the use of gelatin capsules to administer single doses of the compound at 150, 300, 600, 900, or 1,200 mg/kg. They used two control groups, one receiving no treatment and the other receiving empty capsules. There was a 3-week observation period after which sacrificed animals were necropsied and examined histopathologically. The two formulations were closely similar in calculated acute oral LD_{50} values, 596.8 mg/kg for technical HCH and 598.5 mg/kg for technical X-factor.

2.4.1.2 Avian Oral Toxicity – Subchronic

No data are available.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects observed at the LOAEL
Cornacoff et al. 1988	Mice (&) (B6C3F1)	30 d	15(β)	45	Immunotoxicity: Reduced splenic proliferation to PHA, Con-A, and LPS
Ravender et al. 1990	Mice (%) (Swiss)	14 d	NA (tech)	60	Increase in relative liver weight
			NA (γ)	30	Increase in relative liver weight
Barros & Saliba 1978	Rats (Wistar)	90 d	0.045 (tech)	45	Histopathological lesions in liver and kidney
Dikshith et al. 1978	Rats (%) (Unstated)	90 d	NA (γ)	17.6	Liver necrosis and histopathological lesions of the testis
Shivanandappa & Krishnakumari 1981	Rats (%) (CFT-Wistar)	90 d	NA (tech)	5	Increases in relative weights of liver, heart, and kidney
			12.5 (tech)	37.5	Hypertrophy of the liver
Zeocon Corp. 1983	Rats (Wistar KFM-Han SPF)	12 w	0.31 (γ)	1.55	Hypertrophy of the liver; $\alpha_{2\mu}$-globulin-related nephropathy
Oesch et al. 1982	Rats (OM)	3 mo	5.5 (γ)	18	Increase in relative liver weight
	Mice (CF1)	3 mo	16.7(γ)	54	
Muralidhara & Krishnakumari 1983	Rats (CFT-Wistar)	90 d	10.8 (X-factor)	31.8	Histopathological lesions (hypertrophy and hyperplasia) and increased organ weights in the liver, kidney, and testis
Andrews & Gray 1990	Rats (%) (LE)	10 w	NA(γ)	10	Kidney histopathology and functional deficits
Van Velsen et al. 1986	Rats (Wistar)	13 w	NA(β)	0.1	Increase in relative kidney weight
Fitzhugh et al. 1950	Rats (Wistar)	Lifetime	0.1 (β)	0.5	Increase in relative liver weight
			NA(β)	0.5	Increased relative weights of kidney and liver associated with histopathology
			0.5 (β)	5	Testicular atrophy in males
			2.5 (β)	5	Decreased growth of 21-day-old weanlings over first 6 months of study
			0.5 (α)	2.5	Increased relative weights of kidney and liver associated with histopathology
			5.0 (α)	40	Testicular atrophy in males
			2.5 (γ)	5	Increased relative weights of kidney and liver associated with histopathology
			40 (γ)	80	Decreased growth of 21-day-old weanlings over first 6 months of study
			0.5 (tech)	2.5	Increased relative weights of kidney and liver associated with histopathology
			5.0 (tech)	40	Testicular atrophy in males
Rivett et al. 1978	Dogs (Beagle)	104 w	1.6 (γ)	2.92	Liver changes and altered serum alkaline phosphatase activity
Dikshith et al. 1991	Rats (%) (Unstated)	1 yr	0.4 (tech)	2	Increase in relative liver weight
			2.0 (tech)	20	Histopathological changes in liver, kidney, and testis
Palmer et al. 1978	Rabbit (N Zealand)	GD 6-18	20 (γ)	NA (γ)	No effect on reproductive parameters. Unbounded NOAEL
	Rat (CFY)	GD 6-15	20 (γ)	NA (γ)	No effect on reproductive parameters. Unbounded NOAEL
Khera et al. 1979	Rat (Wistar)	GD 6-15	25 (Benesan)	NA (Benesan)	No effects on reproductive parameters. Unbounded NOAEL
Saxena et al. 1986	Rat (IRC)	GD 6-14	20 (γ)	NA (γ)	No effects on reproductive parameters. Unbounded NOAEL

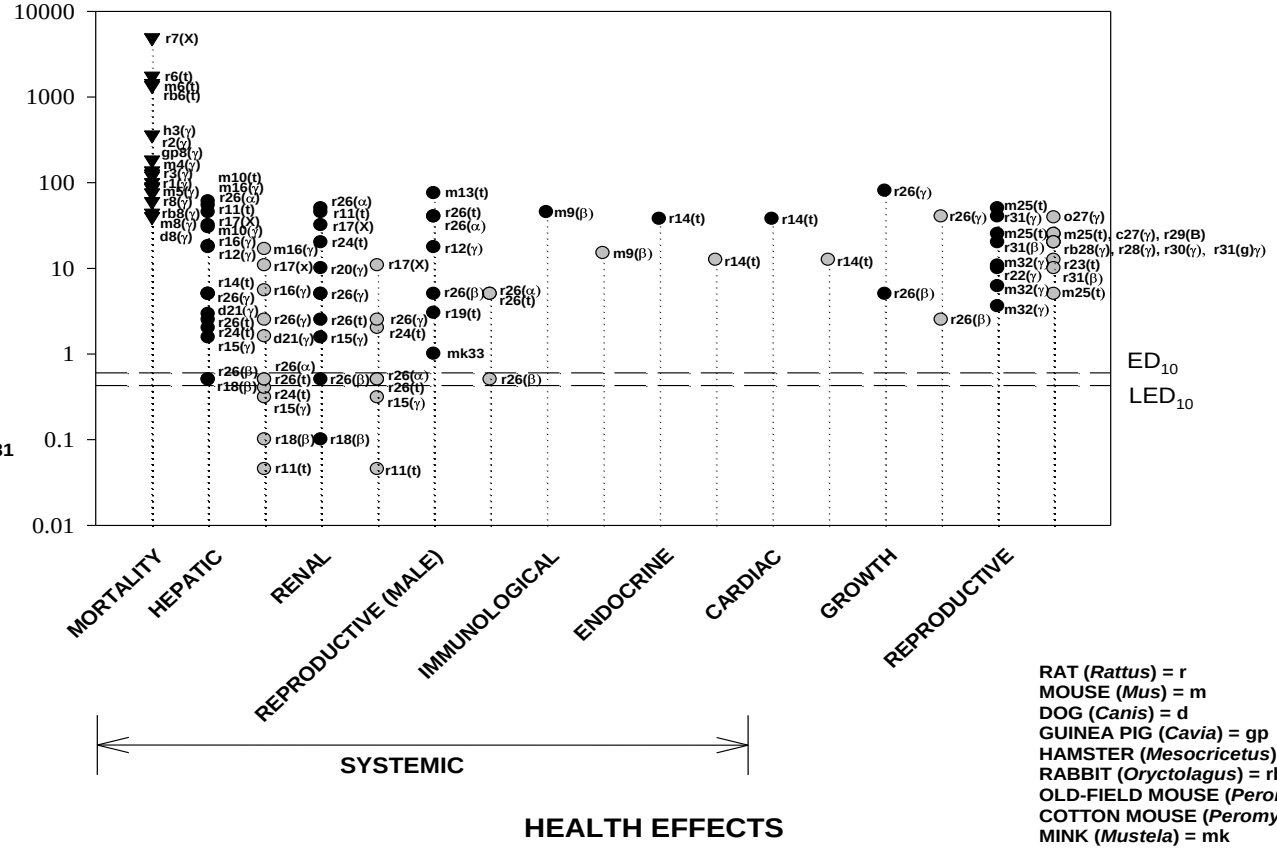
Table 2. Summary of Relevant Mammalian Data for TRV Derivation (continued)

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects observed at the LOAEL
Srinivasan et al. 1991	Rat (Wistar)	Gestation, Gest. + Lactation, or Lact.	10 (β)	20 (β)	Mortality of pups within 28 days of parturition
			20 (γ)	40 (γ)	Reduced fertility and increased pup mortality
Srivastava et al. 1991	Mice	GD 6-12	25 (tech)	50 (tech)	Fetal resorption
Srivastava & Raizada 1993	Mice (Swiss)	GD 6-12	5 (tech)	25 (tech)	Reduced maternal body weight gain.
Sircar & Lahini 1989	Mice (Swiss)	GD 1-4	NA (γ)	10.7 (γ)	Prevented implantation during early pregnancy
		GD 6-12	NA (γ)	6.14 (γ)	Fetal resorption (100%)
		GD 14-19	NA (γ)	3.58 (γ)	Pups died within 5 days of parturition
Ravinder et al. 1990	Mice (%) (Swiss)	14 d	NA (tech)	60	Increase in relative liver weight
			NA (γ)	30	Increase in relative liver weight
Barros & Saliba 1978	Rats (Wistar)	90 d	0.045 (tech)	45	Histopathological lesions in the liver and kidney
Gautam et al. 1989; Chowdhury & Gautam 1990	Rats (%) (Charles Foster)	180 d	NA (tech)	3	Testicular degeneration and other lesions to the male reproductive system
Gray et al. 1988	Rats (Unstated)	105-151 d	NA (γ)	10	Mortality among newborn kits
Wolfe & Esher 1980	Old-Field Mice	8 mo	39 (γ)	NA (γ)	No effects on reproductive parameters. Unbounded NOAEL (UN).
	Cotton Mice	8 mo	24 (γ)	NA (γ)	No effects on reproductive parameters (UN).
Yamasaki et al. 2005	Rats	2-Gen	15 (γ)	ND	No effects on reproductive parameters (UN).
Beard and Rawlings (1998)	Mink	3-Gen	ND	1 (γ)	40% reduction in mean litter size; decrease in thyroid mass, decrease in second matings.
Nigam et al. 1979	Mice (%) (Swiss)	30-40 w	NA (tech)	3	Testicular atrophy and histopathological changes to spermatogonic cells.

NA = Not applicable LE = Long-Evans rats OM = Osborne-Mendel rats
ND = Not determined

Figure 1

HEXACHLOROCYCLOHEXANE: HEALTH EFFECTS TO MAMMALS



1 = Gaines 1969
2 = Boyd and Chen 1968
3 = Truhaut et al. 1974
4 = Meera et al. 1992
5 = Lahiri and Sircar 1991
6 = Dikshith et al. 1989
7 = Krishnakumari et al. 1982
8 = Lewis 1989
9 = Cornacoff et al. 1988
10 = Ravinder et al. 1990
11 = Barros and Saliba 1978
12 = Dikshith et al. 1978
13 = Nigam et al. 1979
14 = Shivanandappa and Krishnakumari 1981
15 = Zeocon Corp. 1983
16 = Oesch et al. 1982
17 = Muralidhara and Krishnakumari 1983
18 = Van Velsen et al. 1986
19 = Gautam et al. 1989; Chowdhury and Gautam 1990
20 = Andrews and Gray 1990
21 = Rivett et al. 1978
22 = Gray et al. 1988
23 = Srivastava and Raizada 2000
24 = Dikshith et al. 1991
25 = Srivastava et al. 1991; Srivastava and Raizada 1993
26 = Fitzhugh et al. 1950
27 = Wolfe and Esher 1980
28 = Palmer et al. 1978
29 = Khera et al. 1979
30 = Saxena et al. 1986
31 = Srinivasan et al. 1991
32 = Sircar and Lahini 1989
33 = Beard and Rawlings 1998

RAT (*Rattus*) = r
MOUSE (*Mus*) = m
DOG (*Canis*) = d
GUINEA PIG (*Cavia*) = gp
HAMSTER (*Mesocricetus*) = h
RABBIT (*Oryctolagus*) = rb
OLD-FIELD MOUSE (*Peromyscus*) = o
COTTON MOUSE (*Peromyscus*) = c
MINK (*Mustela*) = mk

2.4.1.3 Avian Oral Toxicity – Chronic

In a longer-term study, Whitehead et al. (1974) exposed 12 Japanese quail/group to either a control diet or one spiked with 200 ppm γ-HCH (calculated as 22.5 mg/kg-day assuming a food consumption rate of 0.11 kg food/kg BW-day [based on the food ingestion rate and body weight for Japanese quail reported in Sample et al. 1996]) over a 28-week period (2 groups: 28 weeks at control diet or 18 weeks at control diet followed by 10 weeks treated diet). The authors monitored egg production, egg weight, egg shell thickness, calcium content of the egg shells, and the overall surface appearance of the shell as revealed by scanning electron microscopy. However, no differences were observed between exposed and control groups. Because this study occurs over several weeks and during a critical life stage (i.e., egg production), the 22.5 mg/kg-d dose level may be considered a chronic unbounded NOAEL.

Chakravarty et al. (1986) and Chakravarty and Lahiri (1986) examined the effect of γ-HCH on egg laying and eggshell characteristics, respectively, in domestic mallard ducks gavaged for 8 weeks at 20 mg/kg, either daily, three times/week, or twice/week (equivalent to doses of 20 mg/kg-day, 8.57 mg/kg-day, and 5.71 mg/kg-day). The researchers observed eggshell thinning and marked reductions in egg production, clutch size, and vitellogenin and m-RNA production as a result of the first two dosing regimens. These effects were reversed in those animals that also had received an intramuscular injection of stilbestrol. This raises the question of whether lindane may be exerting a developmental effect in birds by inhibiting the synthesis or release of estrogens. Because these studies considered exposure during a sensitive life stage, the 5.71 and 8.57 mg/kg/d doses were considered to be the chronic NOAEL and LOAEL, respectively.

2.4.1.4 Avian Oral Toxicity – Other

The same research group associated with Chakravarty and Lahiri published a research review in which they describe the hematological effects of γ-HCH when administered via a single gavage dose at 5 mg/kg to house sparrows (*Passer domesticus*), baya weavers (*Ploceus philippinus*), common mynas (*Acridotheres tristis*), rose-ringed parakeets (*Psittacula krameri*), blue-necked pigeons, and domestic ducks (*Anas platyrhynchos*) (Lahiri et al. 1990). As described by the authors, γ-HCH induced a reduction of hemoglobin and hematocrit with corresponding changes in mean cell volume, and mean cellular hemoglobin in all exposed birds.

By contrast to the fore-going experimental studies, a report by Blus et al. (1984) discussed the effects of pesticides on Canada geese at the Umatilla National Wildlife Refuge in Oregon that had been exposed to wheat coated with heptachlor epoxide to counteract wireworms. The use of heptachlor resulted in a "die-off" of the geese with the number of successful nests declining as the concentration of heptachlor epoxide in eggs increased. Changing the coating to γ-HCH resulted in increased breeding success among

the geese. Blus et al. (1984) considered that, while γ-HCH can induce fatalities in birds, the threshold concentrations are higher than for some other pesticides, since the compound is less readily accumulated than some other organochlorine pesticides.

Helberg, et al. (2005) studied the impact of organochlorine contaminants on breeding wild great black-backed gulls (*Larus marinus*). While the data were not conclusive because of the presence of other pesticides and possible nutritional issues, the authors noted a positive correlation between blood levels of β-HCH and the probability that a nesting site under the care of a female bird would be predated upon by ravens or hooded crows, resulting in smaller clutch size.

2.4.1.5 Studies Relevant for Avian TRV Development for Ingestion Exposures

Of the avian studies located, only Whitehead et al. (1974), Chakravarty et al. (1986), and Chakravarty and Lahiri (1986) are suitable for TRV derivation. These studies are chronic in nature and evaluate relevant toxic effects such as reproduction (i.e., egg production). Two species included (Japanese quail and the domestic mallard duck) in these studies represent two Avian Orders and two Avian Families: Galliformes: Phasianidae and Anseriformes: Anatidae. An unbounded NOAEL was available for egg production in Japanese quail, whereas both NOAEL and LOAEL values may be derived from the Chakravarty et al. studies.

2.4.2 Avian Inhalation Toxicity

No data are available.

2.4.3 Avian Dermal Toxicity

Studies to evaluate the dermal effects of γ-HCH in birds were not located; however, the effect of direct application to bird eggs have been investigated. Hoffman and Albers (1984) monitored the acute lethality of γ-HCH in embryos by including the substance as one of 42 environmentally important substances applied to the outer shells of fertile mallard eggs. The authors expressed their results in terms of an equivalent amount of pesticide that would be applied in field conditions. Thus, for γ-HCH, their results equate to an LC_{50} of 62 lbs/acre when the compound was applied to the egg in an aqueous emulsion. By contrast, a value of 8.5 lbs/acre was obtained when the compound was applied to the egg in oil. This concentration was considered to be equivalent to an applied dose of 41.5 μg/g egg. Hoffman and Albers (1984) reported that they did not see any decline in growth but did observe the development of visceral abnormalities when γ-HCH was applied to eggs at concentrations below the LC_{50} (see also Hoffman and Eastin 1982).

2.5 Summary of Amphibian Toxicology

Amphibian toxicity data for HCH effects were limited to only three studies. Marchal-Segault and Ramade (1981) carried out a dose response experiment in which spawn of *Xenopus laevis* (African

clawed frog) were incubated in water, water plus 0.1% acetone, or 0.5, 1.0, or 2.0 ppm γ-HCH ppm in 0.1% acetone. The researchers monitored the lethality of γ-HCH to eggs and tadpoles over a period of a week and recorded decreased hatching rates at 2 ppm, although most of the aborted eggs contained well-developed embryos. This suggested that the pesticide's toxicity may have been associated with a disturbance in the hatching mechanism. Once hatched, the mortality of the tadpoles was relatively unaffected by the pesticide during the first week post-hatch. However, by 6 weeks post-hatch, 100 percent mortality was observed in the two highest exposure groups. Although not statistically significant, mortality at the 0.5 ppm exposure level appears elevated compared to controls. Additionally, increased mortality and a 4 week delay in completion of metamorphosis compared to controls was observed at the 0.5 ppm concentration, and those reaching metamorphosis weighed approximately one-half that of the controls. Given these effects in the lowest exposure concentration, 0.5 ppm (0.5 mg/L) may be considered an unbounded LOAEL for growth and development.

Pawar and Katdare (1983, 1984) studied the effect of technical HCH (13% γ-HCH) on the embryonic development of the frog, *Microhyla ornata* (ornate pigmy frog) and developed 96-hour LC_{50}s of 23.37 mg/L for embryos and 7.27 mg/L for tadpoles. As evidenced by the LD_{50} values, the tadpoles were more sensitive than the embryos for this species and developed a number of morphological and physiological deficits. The authors exposed fertilized eggs (embryos) and 8-day-old tadpoles to concentrations of 0, 1, 10, 20, 40, 60, and 70 mg/L for embryos and 0, 1, 20, 20, 40, and 60 mg/L for tadpoles. Clear dose-response relationships were observed for percent hatching after 48 hours, percent abnormalities after 96 hours, and percent mortality after 96 hours. Although the authors do not indicate at which dose these effects become statistically significant, effects become apparent at 10 mg/L and a dramatic decrease in hatching success and increase in abnormalities (e.g., retarded growth and upward and lateral curvature of the tail) and mortality occurred at 20 mg/L. These results suggest a NOAEL of 1 mg/L and a LOAEL of 10 mg/L for developing embryos. Eight-day tadpoles exhibited abnormalities similar to those observed in embryos, including retarded growth, upward curved tail, poor pigmentation, and circular movement and loss of balance. Both studies indicate that the effects on tadpoles were observed at 10 mg/L, though it is suggested that they occur in all treated tadpoles. Additionally, the LC_{50} for tadpoles is stated to be 7.27 mg/L; therefore, it is presumed that sublethal effects were observed at concentrations lower than 7.27 mg/L. Consideration of the authors' comments and the LC_{50} suggests that 1 mg/L may represent an unbounded LOAEL for tadpoles.

2.6 Summary of Reptilian Toxicology

No toxicological data for the effects of hexachlorocyclohexane are available for reptiles.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Studies relevant to the development of oral ingestion TRVs for mammals were discussed in Section 2.3.1.6 and presented in Table 2 and Figure 1. Of these studies, four were evaluated as sensitive representatives of the three most relevant endpoints (liver histopathology, growth, and reproduction). Shivanandappa and Krishnakumari (1981) and Van Velsen et al. (1986) both reported significant dose-response relationships between HCH (technical and β-HCH, respectively) exposure in rats and increased liver weights. Histopathological effects such as liver hypertrophy were also observed in these studies. Using increased liver weight as the relevant endpoint, an unbounded LOAEL of 5 mg/kg-day for technical HCH (13.6 % β-HCH) was indicated by the data in Shivanandappa and Krishnakumari (1981). Van Velsen et al (1986) evaluated lower doses of HCH using only the β-isomer with a resulting NOAEL and LOAEL for the same endpoint of 0.1 and 0.5 mg/kg-day, respectively. The effects of α-, β-, γ- and technical HCH on growth of 28 day-old rat weanlings over the first 6 months of exposure were investigated by Fitzhugh et al. (1950). All isomers and technical HCH retarded growth, with significant reductions observed at 40, 5, 80, and 40 mg/kg-day for α-HCH, β-HCH, γ-HCH, and technical HCH, respectively. The authors reported that no effects on growth occurred at the 0.25 and 2.5 mg/kg-day dose levels for all isomers or formulations; however, the data for these dose groups was not presented. Of the data presented, γ-HCH showed the strongest dose-response relationship; however, it was not the most toxic isomer for this endpoint. The β-HCH was the most toxic isomer with a NOAEL and LOAEL for growth of 2.5 and 5 mg/kg-day, respectively. For effects on reproduction, Srivastava and Raizada (1993) reported reductions in maternal weight gain and number of live fetuses per dam, as well as increases in the number of resorptions and percent loss of implantations from exposure of Swiss mice to technical HCH during gestation days 6 through 12. All four of these endpoints showed a significant dose-response relationship, with percent maternal weight gain being the most sensitive. The NOAEL and LOAEL for effects of technical HCH on maternal weight gain are 5 and 25 mg/kg-day, respectively.

Additionally, Beard and Rawlings (1998) found a reduction in mean litter size in subsequent litters and reported reductions in subsequent female mating acceptance at 1 mg/kg-d in a study that exposed mink to lindane for two generations. However, because only three treatment groups were used, a robust dose-response evaluation (BMDS) could not be performed.

Although the liver is an important organ in rats for the metabolism of carbohydrates and proteins, production of proteins (including those for coagulation factors), and detoxification (part of the immune

system), an enlarged liver by itself is not evidence supportive of adverse health effects. Additionally, the two highest quality studies for this endpoint are considered to be subchronic in duration; and data from the chronic studies are largely supportive and contain relevant data on growth and reproduction. Of these two endpoints, reproduction was the most sensitive; therefore, the study by Srivastava and Raizada (1993) was selected for final TRV derivation. In addition, this and other studies discussed in Section 2.3.1.6 meet the minimum data set requirement of the Standard Practice, Section 2.2 (USACHPPM 2000), and no uncertainty factors are needed to derive the TRVs.

The percent maternal weight gain data from Srivastava and Raizada (1993) were appropriate for a benchmark dose derivation and are presented in Appendix B. A benchmark dose (BMD or ED10) of 0.604 mg technical HCH/kg-day was calculated from the model fit of the mean response at the 10 percent response level. A lower-bound on the benchmark dose (BMDL or LED10) was calculated to be 0.428 mg technical HCH/kg-day from the lower 95 percent confidence interval (CI) of the modeled curve. As indicated in Section 2.3.1.6, a clear difference in toxicity among γ-HCH, technical HCH, and X-Factor was observed in the available LD_{50} data (i.e., γ-HCH was the most toxic). However, trends in toxicity among HCH isomers or formulations within the other studied endpoints were not apparent. Therefore, the TRVs developed from the technical HCH data are considered to represent all isomers and formulations of HCH. As depicted in Figure 1, the derived ED10 and LED10 values for reproduction are protective of the other endpoints (i.e., about 90 % of NOAELs and LOAELs are greater than the TRVs). Generally, those NOAELs and LOAELs that fall below the derived TRVs were considered to be suspect in relevance or accuracy. For example, data from Barros and Saliba (1978) indicated a NOAEL of 0.045 mg/kg-day for hepatic and renal toxicity. However, the LOAEL for these endpoints in the study was 45 mg/kg-day. Because there were four orders of magnitude between dose levels, the applicability of the NOAEL is highly uncertain. Dikshith et al. (1991) observed increased liver weights at 2.0 mg/kg-day, with a corresponding NOAEL of 0.4 mg/kg-day. Because the liver weight data were not presented, TRVs using the benchmark dose method could not be developed from this study. However, it should be noted that both TRVs derived from the reproductive study are less than the LOAEL and the LED10 is nearly equivalent to the NOAEL (0.428 mg/kg-day compared to 0.4 mg/kg-day).

Table 3 presents the selected class-specific TRVs. Results of the many studies evaluated were consistent across HCH isomers and formulations, species, and endpoints and the selected studies were of high quality. Therefore, these TRVs were given a high degree of confidence.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose mg/kg-d	Confidence
LED_{10}	0.428	High
ED_{10}	0.604	High

3.1.2 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, the class-specific TRVs shown in Table 3 may be considered to apply to small herbivorous mammals because mice are members of this guild. As with the class-specific TRVs, only two species are represented so confidence in the TRVs is low to medium. Data to derive TRVs for other guild associations (e.g., carnivorous mammals) is not available at this time.

3.1.3 TRVs for Inhalation Exposures for the Class Mammalia

No data were identified permitting derivation of a mammalian inhalation TRV for hexachlorocyclohexane.

3.1.4 TRVs for Dermal Exposures for the Class Mammalia

Sufficient data required for development of dermal TRVs for Class *Mammalia* were not available. However, an acute LD_{50}s of >8,000 mg/kg of technical HCH for rats and 1,786 mg/kg for white rabbits have been identified. These high LD_{50} values suggest that technical HCH (and possibly individual HCH isomers) has a relatively low dermal toxicity to mammals.

3.2 Toxicity Reference Values for Birds

3.2.1 TRVs for Ingestion Exposures for the Class Aves

As described in Section 2.4.1.5, the three chronic avian studies (Whitehead et al. 1974, Chakravarty et al. 1986, and Chakravarty and Lahiri 1986) were sufficient for TRV derivation. Whitehead et al. (1974) evaluated the reproductive effects of γ-HCH administered at 22.5 mg/kg-day for 10 weeks to Japanese quail. Because no effects were observed at this level, it may be considered an unbounded NOAEL. Chakravarty et al. (1986) and Chakravarty and Lahiri (1986) evaluated the effect of γ-HCH on reproduction in mallard ducks exposed at 5.71, 8.57, or 20 mg/kg-day. Significant reductions in egg production and clutch size were observed at the two highest doses as reported in Chakravarty et al (1986). Chakravarty and Lahiri (1986) reported the effects on eggshell characteristics. The Chakravarty et al.

(1986) study shows a clear dose-response relationship for a relevant toxic endpoint (i.e., egg production); therefore, the NOAEL and LOAEL from this study (5.71 and 8.57 mg/kg-day, respectively) were selected for development of the avian TRVs.

An effect on reproduction is one of the endpoints considered particularly relevant to the health and ecology of the whole organism in the Standard Practice (USACHPPM 2000). Because the NOAEL and LOAEL from Chakravarty et al. (1986) are lower than the NOAEL determined in Whitehead et al. (1974), this study is protective of the only other species evaluated. Although two avian Orders are represented, only two species are included in the three relevant studies. Therefore, the minimum data set requirements of the Standard Practice, Section 2.2 (USACHPPM 2000) were not met and TRVs for Class Aves were based on an approximation of the NOAEL and LOAEL using Uncertainty Factors (UF). A UF of 10 was applied to the NOAEL and LOAEL (5.71 and 8.57 mg/kg-day, respectively) from Chakravarty et al. (1986) to account for interspecific variability. Table 4 presents the selected TRVs. A low level of confidence has been given to these TRVs because the experimental design in each of the three studies was considered to be moderate in quality (i.e., one study had only one dose level and the other studies obtained different daily dose levels only by varying the weekly dosing regime). Additionally, only studies relating effects to exposure to γ-HCH were located; therefore, the relative toxicity of γ-HCH to other HCH isomers or HCH formulations (e.g., X-factor or technical) could not be evaluated.

Table 4. Selected Ingestion TRVs for the Class Aves

TRV	Dose mg/kg-d	Confidence
NOAEL-based	0.571	Low
LOAEL-based	0.857	Low

3.2.2 TRVs for Ingestion Exposures for Avian Foraging Guilds

TRVs specific to particular guild associations (e.g., herbivorous birds) have not yet been derived. However, the class-specific TRVs shown in Table 4 may be considered to apply to primarily herbivorous birds, though the confidence in these TRVs is low. Data to derive TRVs for other guild associations (e.g., carnivorous birds) is not available at this time.

3.2.3 TRVs for Inhalation Exposures for the Class Aves

No data were identified permitting derivation of an avian inhalation TRV for hexachlorocyclohexane.

3.2.4 TRVs for Dermal Exposures for the Class Aves

No data on the dermal toxicity of HCH on birds is available at this time. It should be noted that the study in which γ-HCH was painted onto the shells of fertile mallard eggs was located (Hoffman and Albers 1984). For this study, an LC_{50} (median lethal concentration) of 41.5 mg/kg egg was obtained. This suggests that γ-HCH can be absorbed through the eggshell, and that it is moderately toxic to developing embryos. This study is not sufficient for development of dermal TRVs.

3.3 Toxicity Reference Values for Amphibians

The effects of both γ-HCH and technical HCH on amphibians (e.g., reduced hatching success, retarded growth in tadpoles) were consistent across the three studies and two species (*Xenopus laevis* and *Microhyla ornate*) evaluated. These two species represent one amphibian Order and two Families: Anura: Pipidae and Microhylidae. For each species, it was found that the tadpole stage was more sensitive than the embryo stage, although effects were observed during both sensitive life stages. Marchal-Segault and Ramadeither (1981) observed increased mortality, delayed metamorphosis, and retarded growth in tadpoles after 6 weeks exposure to 0.5 mg/L γ-HCH. Similarly, Pawar and Katdare (1983, 1984) reported retarded growth and other abnormalities (upward curved tail, poor pigmentation, and circular movement with loss of balance) in 8-day tadpoles treated with technical HCH (presumably these effects occurred at the lowest treatment group of 1 mg/L). Because these studies were conducted during sensitive life stages, they are considered to be chronic. Additionally, effects on reproduction, growth, and survival are directly relevant to the health and ecology of the whole organism, and are considered appropriate endpoints for TRV derivation. As the lowest effect level, the unbounded LOAEL (0.5 mg/L) from Marchal-Segault and Ramade (1981) was selected for development of the TRVs.

Because only one Order and two species were evaluated in the relevant studies, the minimum data set requirements of the Standard Practice, Section 2.2 (USACHPPM 2000) were not met. Therefore, TRVs based on an approximation of the NOAEL and LOAEL were developed for amphibians using UFs. An UF of 10 was applied to the LOAEL (0.5 mg/L) from Marchal-Segault and Ramade (1981) to account for interspecific variability. A chronic NOAEL was estimated by applying an UF of 10 to the chronic LOAEL. Table 5 presents the selected TRVs. A low to medium level of confidence has been given to these TRVs because effects were highly consistent across two species, but a NOAEL for these endpoints (as determined within the experimental design of the available studies) was unavailable.

Table 5. Selected Ingestion TRVs for Amphibians

TRV	Exposure mg/L	Confidence
NOAEL-based	0.005	Low to Medium
LOAEL-based	0.05	Low to Medium

3.4 Toxicity Reference Values for Reptiles

Not available at this time

4. IMPORTANT RESEARCH NEEDS

Mammalian TRVs derived for HCH have a high confidence level because many studies have been conducted utilizing several species (including 2 wildlife species) and representing a variety of toxicological endpoints. If any additional studies are conducted, these should evaluate the reproductive, developmental, or growth effects of HCH on additional wildlife species using multiple dose levels to achieve NOAELs and LOAELs for the evaluated endpoints. Inhalation and dermal studies on mammals were either limited or lacking for HCH. TRV derivation for birds, amphibians, and reptiles was more uncertain than for mammals, due to the paucity of high quality toxicity data for birds, the lack of studies with sufficiently low dose levels to determine a NOAEL for amphibians, and the absence of toxicity data for reptiles. Before reliable avian, amphibian, and reptilian TRVs can be derived, HCH toxicity to these classes of wildlife needs to be adequately characterized. Appropriate acute, subacute, subchronic and especially chronic HCH toxicity data for birds, amphibians, and reptiles, by all exposure routes, are needed. The research studies should include experimental models of species genetically, biologically and behaviorally close to wildlife most likely to be exposed, and the experimental design should mimic both exposure type and duration, and include assessments of long-term effects.

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APPENDIX A
LITERATURE REVIEW

On 6 June 2001, the following files were searched in DIALOG:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 35 Dissertation Abstracts, File 76 Life Sciences Collection, and File 185 Zoological Record.

The search strategy for **Amphibians & Reptiles**:

- The expressions hexachlorocyclohexane, lindane and BHC and the CAS numbers for the following isomers/formulations: technical HCH, α-HCH, β-HCH, γ-HCH, δ-HCH, and ε-HCH
- AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- AND (reproduc? or dietary or systemic or development or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD (reduce duplicates)

The search strategy for **Birds:**

- The expressions hexachlorocyclohexane, lindane and BHC and the CAS numbers for the following isomers/formulations: technical HCH, α-HCH, β-HCH, γ-HCH, δ-HCH, and ,ε-HCH
- AND chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- AND (reproduc? or dietary or systemic or development or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD (reduce duplicates)
- NOT (human? or culture? or (cell()line) or gene or vitro or inject or subcutane? or skin? or cancer? or salmonella or carcin? or tumo?)
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)

The search strategy for **Wild Mammals:**

- The expressions hexachlorocyclohexane, lindane and BHC and the CAS numbers for the following isomers/formulations: technical HCH, α-HCH, β-HCH, γ-HCH, δ-HCH, and ,ε-HCH
- AND (didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or

mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?

- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- RD (reduce duplicates)
- NOT (human? or culture? or (cell()line) or gene or vitro or inject or subcutane? or skin? or cancer? or salmonella or carcin? or tumo?)
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)

The search strategy for **Laboratory Mammals:**

- The expressions hexachlorocyclohexane, lindane and BHC and the CAS numbers for the following isomers/formulations: technical HCH, α-HCH, β-HCH, γ-HCH, δ-HCH, and ε-HCH
- AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- RD (reduce duplicates)

The strategy outlined above yielded 43 hits for HCH with reptiles/amphibians, 173 articles with birds, 99 with wild mammals and 688 articles with laboratory mammals.

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

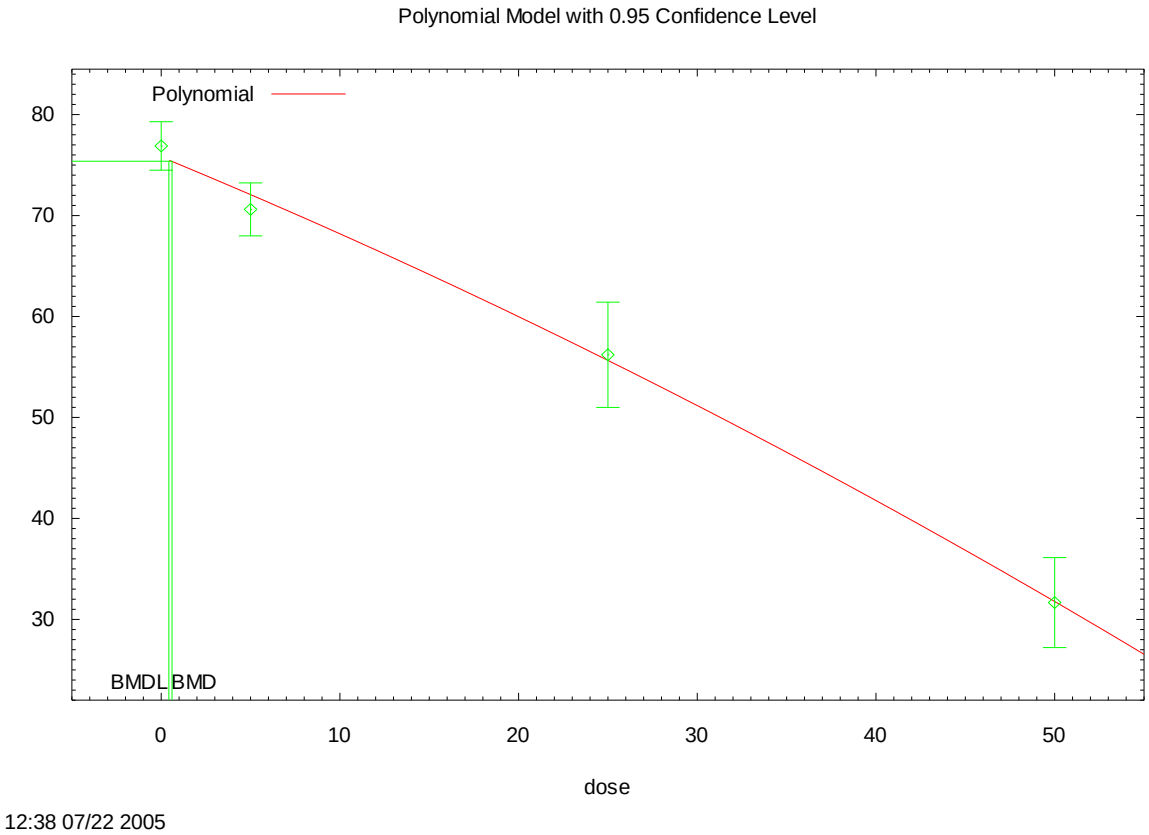
As noted above and in Section 2.1, 1003 hits on chlordane were obtained in the initial search, of which 129 were selected (Tier 2) as being relevant to this survey of the impacts of chlordane on wildlife.

During the period 25-30 July 2007, additional database searches were performed to update available information. A search on the EPA ECOTOX database using the chemical search terms lindane, BHC, and hexachlorocyclohexane in both plant and animal kingdoms yielded no new data for the period 2001-2007 that was relevant to the report. A search of

APPENDIX B
Benchmark Dose Calculation for Mammals

Benchmark dose methodology was developed by the EPA in order to determine a point of departure (POD) dose for assessment of non-cancer health effects. The benchmark dose (BMD) corresponds to the probability (usually 5% or 10%, expressed as ED_5 or ED_{10}, respectively) that the chosen effect will be observed in a population. The BMD is intended to replace the NOAEL/LOAEL-approach for determining the impact of exposure dose on a population. In order to use the BMD-approach, the data must exhibit a dose-response trend, and preferably have multiple points near the lower end of the response spectrum so that minimum confidence limits can be obtained at the benchmark dose.

The data presented below are from Srivastava and Raizada (1993) with percent maternal body weight gain during gestation in Swiss mice as the response. These data showed a clear dose-response relationship and are protective of other relevant endpoints. The model fit was adequate, and a benchmark dose (BMD) and a benchmark dose lower confidence limit (BMDL) were obtained from this analysis.



The form of the response function is:

Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = MEAN

Independent variable = DOSE

rho is set to 0

Signs of the polynomial coefficients are not restricted

A constant variance model is fit

Total number of dose groups = 4

Total number of records with missing values = 0

Maximum number of iterations = 250

Relative Function Convergence has been set to: 1e-008

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha =	21.3608
rho =	0 Specified
beta_0 =	75.8225
beta_1 =	-0.731023
beta_2 =	-0.00299311

Parameter Estimates

95.0% Wald Confidence Interval

Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
alpha	19.5998	4.89995	9.99607	29.2035
beta_0	75.8225	1.29703	73.2804	78.3647
beta_1	-0.731023	0.158862	-1.04239	-0.419659
beta_2	-0.00299311	0.00306624	-0.00900283	0.00301661

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	beta_0	beta_1	beta_2
alpha	1	-9.4e-013	-1.6e-013	-1.3e-014
beta_0	-9.4e-013	1	-0.65	0.51
beta_1	-1.6e-013	-0.65	1	-0.97
beta_2	-1.3e-014	0.51	-0.97	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2
0	8	76.9	2.87	75.8	4.43	0.682
5	8	70.6	3.14	72.1	4.43	-0.947
25	8	56.2	6.24	55.7	4.43	0.341
50	8	31.7	5.33	31.8	4.43	-0.0758

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-62.848380	5	135.696759
A2	-59.486428	8	134.972855
fitted	-63.608295	3	133.216590
R	-108.853825	2	221.707650

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)

Test 2: Are Variances Homogeneous (A1 vs A2)

Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	98.7348	6	<.0001
Test 2	6.7239	3	0.08124
Test 3	1.51983	1	0.2176

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 0.604118

BMDL = 0.428423

BMDL computation failed for one or more point on the BMDL curve. The BMDL curve will not be plotted